Target ID:MYO5BA
Entry Clone ID: MYO5BA-s001
Allele ID: MYO5BA-a002Â Â Â Â Â Â Â Â Â Â Â Â
Construct IDÂ MYO5BA-c002
Clone IDÂ MYO5BA-k002
Expression IDÂ MYO5BA-e007
Purification ID:Â MYO5BA-p003
Entry clone source:Â MGCÂ Â Â Â Â Â Â Â Â Â Â Â Â Â Â Â Â Â Â
Vector:Â Brazil-1-pNIC28-Bsa4
***E.coli* strain**: BL21(DE3)-R3-pRARE2Â Â Â
Tags and additions:Â N-terminal, TEV protease cleavable hexahistidine tag, tag not been cleaved

Coding DNA sequence

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ATGAAGAAAGCCCAGGACCTAGAAGC
TGCCCAGGCATTGGCCCAGAGTGAGA
GGAAGCGCCATGAGCTCAACAGGCAG
GTCACGGTCCAGCGGAAAGAGAAGGA
TTTCCAGGGCATGCTGGAGTACCACA
AAGAGGACGAGGCCCTCCTCATCCGG
AACCTGGTGACAGACTTGAAGCCCCA
GATGCTGTCGGGCACAGTGCCCTGTC
TCCCCGCCTACATCCTCTACATGTGC
ATCCGGCACGCGGACTACACCAACGA
CGATCTCAAGGTGCACTCCCTGCTGA
CCTCCACCATCAACGGCATTAAGAAA
GTCCTGAAAAAGCACAATGATGACTT
TGAGATGACGTCATTCTGGTTATCCA
ACACCTGCCGCCTTCTTCACTGTCTG
AAGCAGTACAGCGGGGATGAGGGCTT
CATGACTCAGAACACTGCAAAGCAGA
ATGAACACTGTCTTAAGAATTTTGAC
CTCACCGAATACCGTCAGGTGCTGAG
TGACCTTTCCATTCAGATCTACCAGC
AGCTCATTAAAATTGCCGAGGGCGTG
TTACAGCCGATGATAGTTTCTGCCAT
GTTGGAAAATGAGAGCATTCAGGGTC
TATCTGGTGTGAAGCCCACCGGCTAC
CGGAAGCGCTCCTCCAGCATGGCAGA
TGGGGATAACTCATACTGCCTGGAAG
CTATCATCCGCCAGATGAATGCCTTT
CATACAGTCATGTGTGACCAGGGCTT
GGACCCTGAGATCATCCTGCAGGTAT
TCAAACAGCTCTTCTACATGATCAAC
GCAGTGACTCTTAACAACCTGCTCTT
GCGGAAGGACGTCTGCTCTTGGAGCA
CAGGCATGCAACTCAGGTACAATATA
AGTCAGCTTGAGGAGTGGCTTCGGGG
AAGAAACCTTCACCAGAGTGGAGCAG
TTCAGACCATGGAACCTCTGATCCAA
GCAGCCCAGCTCCTGCAATTAAAGAA
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GAAAACCCAGGAGGACGCAGAGGCTA
TCTGCTCCCTGTGTACCTCCCTCAGC
ACCCAGCAGATTGTCAAAATTTTAAA
CCTTTATACTCCCCTGAATGAATTTG
AAGAACGGGTAACAGTGGCCTTTATA
CGAACAATCCAGGCACAACTACAAGA
GCGGAATGACCCTCAGCAACTGCTAT
TAGATGCCAAGCACATGTTTCCTGTT
TTGTTTCCATTTAATCCATCTTCTCT
AACCATGGACTCAATCCACATCCCAG
CGTGTCTCAATCTGGAATTCCTCAAT
GAAGTCTGA

Final protein sequence

mhhhhhhssgvdlgtenlyfq*smLN
RQVTVQRKEKDFQGMLEYHKEDEALL
IRNLVTDLKPQMLSGTVPCLPAYILY
MCIRHADYTNDDLKVHSLLTSTINGI
KKVLKKHNDDFEMTSFWLSNTCRLLH
CLKQYSGDEGFMTQNTAKQNEHCLKN
FDLTEYRQVLSDLSIQIYQQLIKIAE
GVLQPMIVSAMLENESIQGLSGVKPT
GYRKRSSSMADGDNSYCLEAIIRQMN
AFHTVMCDQGLDPEIILQVFKQLFYM
INAVTLNNLLLRKDVCSWSTGMQLRY
NISQLEEWLRGRNLHQSGAVQTMEPL
IQAAQLLQLKKKTQEDAEAICSLCTS
LSTQQIVKILNLYTPLNEFEERVTVA
FIRTIQAQLQERNDPQQLLLDAKHMF
PVLFPFNPSSLTMDSIHIPACLNLEF
LNEV

Sequence MHHHHHHSSGVDLGTENLYFQ*SM is the purification tag (lower case) plus TEV protease recognition site (*) .

Expression

Expression strain

BL21(DE3)-R3-pRARE2

Transformation

The construct DNA was transformed into competent cells of the expression strain by a standard heat shock procedure.

Glycerol stock preparation

One colony from the transformation was used to inoculate 1 ml of LB media containing 50 Âµg/ml kanamycin and 34 Âµg/ml chloramphenicol, which was placed in a 37Â°C shaker overnight. The next day glycerol stocks were prepared from this overnight culture.

Expression

5 ml glycerol stock were used to inoculate 50 ml of LB media containing 50 Âµg/ml kanamycin and 34 Âµg/ml chloramphenicol, which was placed in a 37Â°C shaker overnight.Â The next day this starter culture was used to inoculate 6L of LB media (1 ml starter culture used per 1L) containing 50 Âµg/ml kanamycin. When the OD600Â reached approximately 1.0 the temperature was reduced to 18Â°C and the cells were induced by the addition of 0.2 mM IPTG. The expression was continued overnight at 18Â°C.

Cell harvest

Cells were harvested by centrifugation at 6500 rpm for 11 min at 4Â°C after which the supernatant was poured out and the cell pellet placed in a -20Â°C freezer.

Purification

Cell Lysis

Cell pellets from 2 liter expression were slowly thawed on ice. Afterwards the cell pellets were dissolved in approximately 80 ml binding buffer and broken by sonication for 15 min with on/off settings of 10 sec and 35% amplitude. After lyses the solids were separated from the supernatant by centrifugation at 4Â°C for 60 min at 50,000 xg. The clear supernatant was transfered to a fresh 50 ml Falcon tube for further purification.

binding buffer: 25 mM Hepes pH 7.5, 500 mM NaCl, 5% Glycerol, 10 mM Imidazole, 0.5 mM TCEP, 1:500 Protease inhibitor cocktail Set III

Column 1

Ni-NTA (4 ml volume in a gravity-flow column).

The clarified cell extract was further purified on a 4 ml of Ni-NTA column. The supernatant, already containing binding buffer, was applied on the column twice before washing and eluting. During the washing step small 5 ml portions of washing buffer were added ten times consecutively.Â The protein was eluted with four times 5 ml of Elution Buffer.

Binding Buffer: see above

Wash Buffer: 20 mM Hepes pH 7.5, 500 mM NaCl, 5% Glycerol, 30 mM Imidazole

Elution Buffer: 20 mM Hepes pH 7.5, 500 mM NaCl, 5% Glycerol, 500 mM Imidazole

Column2

Superdex 200 10/300 column

The wash buffer fractions and elution buffer fractions from column 1 were pooled separately and concentrated to 5 ml with a 30 kDa mwco spin concentrator. The column had been pre-equilibrated with gel filtration buffer at 1.0 ml/min. The 5 ml protein sample was injected onto the column and 1.0 ml fractions were collected. The protein eluted at between 85 ml and 100 ml volume.

Gel Filtration Buffer: 10 mM HEPES, pH 7.5; 200 mM NaCl; 5% Glycerol; 0.5 mM TCEP

Concentration

The eluted protein was concentrated to 10.7 mg/ml and stored at -80Â°C.

Crystallization

Before crystallization protein was treated with Trypsin to allow mild tryptic digest (10mg/mLÂ ofÂ Trypsin per 1mg/mLÂ ofÂ protein). Crystals were grown by vapour diffusion in sitting drop at 4Â°C. by setting up 12.6 mg/ml of protein in the presence of 5 mM NADP+. Crystals appeared in a sitting drop consisting of 100 nl protein and 50 nl well solution which had been equilibrated against 20 ml well solution containing 0.2 M sodium nitrate and 20% (w/v) PEG 3350. Crystals were mounted in the presence of 25% ethylene glycol and flash cooled in liquid nitrogen.Â Â

Data collection

Resolution:Â 2.25 Ã Â

X-ray source: Diamond Light Source beamline IO2